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STUDIES IN THE PRODUCTION OF GRAFTED EMBRYOS.

A. J. GOLDFARB.

(*From the Biological Laboratories, College of the City of New York.*)

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INTRODUCTION.

It is now generally known that the blastomeres of certain kinds of eggs may, after their complete separation, develop into small though otherwise perfect, larvæ. The reverse experiment of reuniting partially separated or even completely separated blastomeres has also been successfully performed. These experiments demonstrated that it is not only possible to derive two embryos from a single egg, but that two or more blastomeres may be more or less recombined into one organism. These results suggested the possibility of grafting not only the blastomeres, but the eggs themselves. Metchnikoff ('86), Morgan ('95), Zur Strassen ('98), Herbst ('00), discovered and described embryos and larvæ which indicated a grafting of several eggs.

The first successful attempt to graft eggs together was made by Driesch in 1896 with the eggs of various European echinoderms. In the course of several years' experimentation, he perfected the method by which he produced agglutinated and fused eggs, about twenty to the thousand. More recently,

H. V. Wilson ('11), Müller ('11), and others demonstrated that somatic cells could also be fused.

Several investigators have repeated Driesch's experiments with the echinoderms found on this side of the Atlantic but without success. By slightly modifying Driesch's method, I finally succeeded in agglutinating and fusing the eggs of *Arbacia punctulata* at Woods Hole, Mass., in relatively large numbers, ten to forty in every hundred.

In this paper, I propose to give a detailed account of the method used in successfully fusing *Arbacia* eggs, to state briefly the effect of the treatment upon normal development, and to describe some of the agglutinated and fused embryos and larvæ. Since Driesch has given so full, so clear and accurate an account of fused larvæ, I will in this paper emphasize the earlier developmental stages and state but briefly in how far the *Arbacia* larvæ are like or unlike the European fusions described by Driesch.

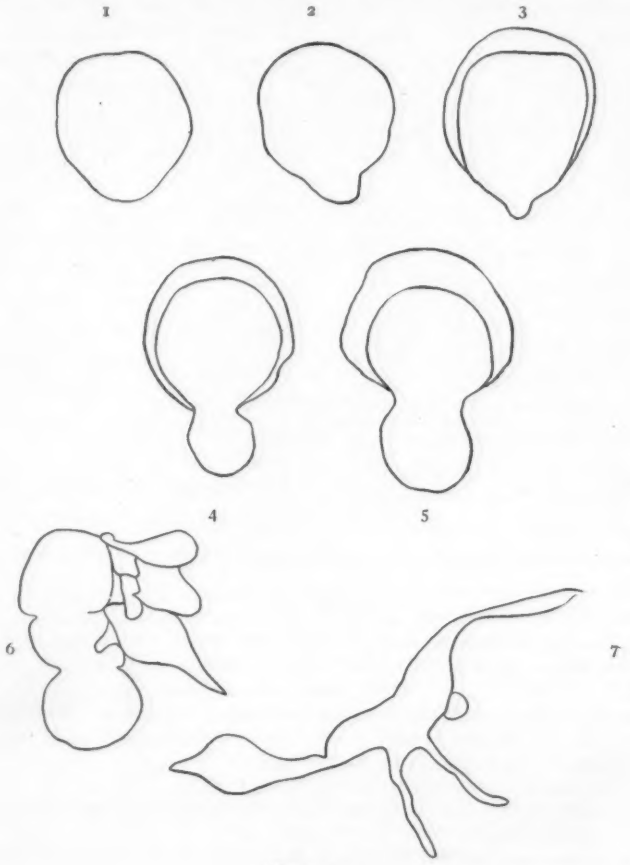
Method Used to Agglutinate Eggs.

The eggs of *Arbacia punctulata* were shaken violently two to three minutes after fertilization so as to remove their fertilization membranes. They were then placed in a calcium-free sea water (van't Hoff formula) prepared with copper or glass distilled water. To this solution four to twelve drops of 0.5 per cent. NaOH were added to every 200 c.c. of the solution. The eggs remained in this alkaline solution for varying periods, as a rule not longer than the first cleavage. Up to this point the method is the one used by Driesch, a method which failed altogether to agglutinate the *Arbacia* eggs. They were then transferred to narrow bore tubes, about 1/8 inch inside diameter, and centrifuged for three to five minutes at about 30 revolutions per minute, and finally placed in sea water. The cultures so treated later contained the agglutinated and fused embryos and larvæ.

As it seemed probable that the violence of the treatment might produce atypic results, a preliminary examination was made to ascertain to what extent anomalies were present in the cultures and how far these were the result of the technique used.

Examination of the Effect of the Treatment upon Development.

Shaking of the Eggs.—When the eggs were shaken about two minutes after fertilization, the fertilization membranes were cast off most of the eggs. When the eggs were shaken four or more minutes after fertilization, the membranes were removed



Figs. 1–7.

with difficulty and the eggs were considerably distorted and otherwise injured. When shaken less than one and a half

minutes after fertilization, many did not segment at all or cleavage was irregular. It was evident that when eggs were shaken about two minutes after fertilization, the fertilization membranes were cast off most readily, and with the least disturbance to subsequent development.

The minimum shaking found necessary to remove the fertilization membranes from all or nearly all the eggs was about one and a half minutes. Figs. 1 and 2, drawn from life immediately after the shaking, represent the eggs freed from their membranes. In Figs. 3, 4 and 5, the eggs have only partially protruded from their membranes. Such extruded parts varied considerably in size, and were often equal to the part within the membrane, Fig. 5. Such "extra-ovates" were the same in kind, as those produced by Loeb' 94, with dilute solutions of sea water. When the egg was divided into two equal or nearly equal parts, twin embryos were formed which often reunited into a more or less single larva. When the extra-ovate was small, as in Figs. 3 and 4, it was most frequently sloughed off and developed into an atypic blastula or, in some instances, an atypic gastrula. Practically all the eggs were elongated but this change of shape was only temporary, and subsequent development was normal.

The rate of development was unaffected by the treatment. This may be seen in Table I. in which the rate of development in each culture is compared with its corresponding control. It is seen that in certain of the experiments the eggs developed more

TABLE I.

The eggs were violently shaken for about two minutes. Their rate of development when compared with the controls showed no material difference.

No.	Result.
20	Shaken lot developed at about the same rate as the controls. Many never reached the pluteus stage.
12*a*	Shaken lot never reached the pluteus stage.
b	Shaken lot behaved like the controls.
37	Shaken eggs developed faster, and more of them reached the pluteus stage, than the controls.
15	Shaken eggs were like the controls, lived longer, but contained more atypic larvæ.
14	Shaken and control lots the same.
25	Shaken and control lots the same. More small-sized larvæ in shaken lot.
34	Shaken and control lots the same. More small-sized larvæ in shaken culture.

slowly or more quickly than its control, but taken altogether the rate of development was clearly unaffected by the shaking; and irregular development was absent, except for the larger number of small atypic blastulæ and gastrulæ arising from the sloughed off small extra-ovates.

Centrifuged Eggs.

When fertilized eggs were centrifuged with or without previous removal of the fertilization membranes, the normality of development depended upon the centrifugal power employed. In Table II., the effect of centrifugalizing from 35 to 90 revolutions per minute, and from 1 to 15 minutes duration, is shown.[1] When centrifuged at the rate of 35 revolutions per minute for ½ to 7½ minutes (in one experiment for 10 minutes), the subsequent development was normal. When centrifuged 60 or more revolutions a minute for even 1 minute considerable numbers of eggs developed but very atypically. When centrifuged for longer periods at this rate, the entire culture was rendered abnormal. At 90 revolutions a minute the embryos were nearly all atypic even though centrifuged ½ to 1 minute. Beyond this rate, or for longer periods of time, the eggs were either killed outright or broken into small fragments, only some of which developed into the atypic blastulæ or atypic gastrulæ already mentioned. These facts are shown in tabular form in Table II.

Table II.

Fertilized eggs were centrifuged at different rates and for varying periods of time. Within well-defined limits of speed and time the embryos and larvæ were quite normal.

No.	Experiment.		Result.
10e	Centrifuged and shaken for 24 minutes.		Few alive. Developed to young plutei only.
20–1	Centrifuged 35 × per min. for	6 min.	Developed to gastrulæ only.
		8	Developed to gastrulæ only.
		10	Developed to gastrulæ only.
		12½	Developed to gastrulæ only.
		15	Developed to blastulæ only. Irregular.
25c	Centrifuged 35 × per min. for	1	Developed normally.
		3	Developed normally.
		5	Developed normally.
		8	Developed irregularly.

[1] The arms of the centrifuge measured 150 mm. each.

TABLE II.—*Continued.*

No.	Experiment.		Result.
33	Centrifuged 35× per min. for	5	Developed normally. Some irreg.
		7½	Developed normally. Some irreg.
		10	Developed normally. More irreg.
38	Centrifuged 35× per min. for	1	Developed normally. More irreg.
		2	Developed normally.
		2	Developed normally.
		3	Developed normally.
		5	Developed normally. More irreg.
		5½	Developed normally. More irreg.
		6	Developed normally. More irreg.
		7	Developed normally. More irreg.
27*c*	Centrifuged 35× per min. for	6	Developed normally. More irreg.
20–2	Centrifuged 60× per min. for	6	Developed to young plutei only. Many irreg.
		8	Developed same.
		10	Developed same.
		13	Developed irregularly.
25–10	Centrifuged 60× per min. for	½	Developed normally.
		1	Developed increasingly irreg.
		2	Developed increasingly irreg.
		4	Developed increasingly irreg.
		5	Developed increasingly irreg.
38*b*	Centrifuged 60× per min. for	2	Developed to gastrula. Most dead.
18	Centrifuged 60× per min. for	1	Developed normally. Some irreg.
		2	Developed normally. Some irreg.
		4	Developed normally. Some irreg.
		6	Developed normally. Many irreg.
		8	Developed normally. Most irreg.
		10	Developed normally. Most irreg.
25–20	Centrifuged 90× per min. for	½	Developed young plutei only.
		1	Developed increasingly irreg.
		2	Developed increasingly irreg.
		3	Developed increasingly irreg.
		4	Developed increasingly irreg.
		5	Developed increasingly irreg.

In an effort to agglutinate the eggs, they were centrifuged not in urine tubes but in finely drawn glass capillaries, plugged at one end with paraffin, and the eggs liberated a few minutes to 24 hours after centrifuging. This method was unsatisfactory, first, because development ceased altogether within the capillaries, and when liberated, after four or more hours, development was atypical. When liberated, after 18 or more hours in the capillaries, they did not develop at all, Table III. Secondly, in removing the eggs, it was necessary to exert considerable force,

enough to separate the eggs from one another. The difficulty was overcome by using narrow bore tubes whose inside diameter was about 3 mm. Eggs centrifuged in these tubes within the centrifugal limits already mentioned, gave rise to normal embryos and larvæ many of which were agglutinated or fused.

TABLE III.

The effect of centrifuging eggs in capillary tubes, after they have been fertilized, and had their membranes removed.

No.	Experiment.	Result.
30–2	Not centrifuged.	Kept in capillary ½ hr. Same as control.
3	Not centrifuged.	Kept in capillary 1 hr. Same as control.
4	Not centrifuged.	Kept in capillary 4½ hrs. Same as control. Clusters present.
5	Not centrifuged.	Kept in capillary 18 hrs. Most dead.
25–30	Centrifuged 30 revol. a min. for 1 min.	15 min. Norm. Clusters present.
31	Centrifuged 30 revol. a min. for 1 min.	30 min. Norm. Clusters present.
32	Centrifuged 30 revol. a min. for 1 min.	60 min. Norm. and irregularities.
33	Centrifuged 30 revol. a min. for 1 min.	90 min. Norm. and irregularities.
40	Centrifuged 60 revol. a min. for 1 min.	15 min. Norm. and irregularities.
41	Centrifuged 60 revol. a min. for 1 min.	30 min. Norm. and irregularities.
42	Centrifuged 60 revol. a min. for 1 min.	60 min. Norm. and irregularities.
43	Centrifuged 60 revol. a min. for 1 min.	90 min. Norm. and irregularities.
50	Centrifuged 90 revol. a min. for 1 min.	15 min. Norm. and irregularities.
51	Centrifuged 90 revol. a min. for 1 min.	30 min. Norm. and irregularities.
52	Centrifuged 90 revol. a min. for 1 min.	60 min. Nearly all dead.
53	Centrifuged 90 revol. a min. for 1 min.	90 min. Nearly all dead.
18–29	Not centrifuged.	Kept in capillary 1 hr. Norm.
30	Not centrifuged.	Kept in capillary 2½ hrs. Norm.
31	Not centrifuged.	Kept in capillary 3 hrs. Plutei irreg.
32	Not centrifuged.	Kept in capillary 5 hrs. Few norm. plutei.
33	Not centrifuged.	Kept in capillary 7 hrs. Only gastrula.
34	Not centrifuged.	Kept in capillary 8 hrs. Died early cleavage.
35	Not centrifuged.	Kept in capillary 19 hrs. Died early cleavage.

CALCIUM-FREE SEA WATER.

When eggs with their membranes removed were placed in calcium-free sea water for half an hour, the blastomeres of the two-cell stage separated from one another either completely,

and the resulting larvæ were perfect though small, or incompletely, and twin or fused plutei were produced as described by Loeb, Morgan and Driesch. When the eggs were kept in the solution for one hour, each of the four quarter blastomeres developed, some into dwarf plutei, others never beyond the gastrula stage. With increasing exposure to the calcium-free sea water, the increasing diminution in the size of the blastomeres, and the probable increasing segregation of formative stuffs, plutei were no longer formed, and abnormal embryos were correspondingly more numerous, most of which died early.

Solutions made with tap water (Woods Hole, Mass.) were highly injurious, for few eggs developed into normal larvæ. With distilled water practically all were normal, provided the eggs were not left in the solution longer than about thirty minutes as shown in Table IV.

TABLE IV.

The effect of calcium-free sea water upon the development of fertilized eggs.

No.	Experiment.		Result.
26*b* 10	Left in solution.	15 min.	Dead. Accident.
11		30	Same as controls and small plutei.
12		45	More small plutei and non-developing blastulæ.
13		60	Only small plutei. Very many irreg.
14		90	Few small plutei. Most irreg. on bottom, dead.
15		120	Few small plutei. Most irreg. on bottom, dead.
	Membranes removed.		
1	Left in solution.	15	Norm. and small plutei.
2		30	Dead. Accident.
3		65	Norm. and small plutei.
4		60	Dead. Accident.
5		90	Plutei and irregular clusters.
6		105	Some plutei, most irregular, clusters.
	Distilled Water.		
18 24	Left in solution.	½ hr.	Norm. and small plutei.
25		1	Small plutei and irregular plutei.
26		2½	Few small plutei. Most irregular.
27		6	Most irreg. Nearly all dead.
28			Most irreg. Nearly all dead.
	Tap water.		
17	Left in solution.	½ hr.	Few norm. plutei.
18		1	Irregular plutei and irregular gastrulæ.
19		2½	Only blastulæ irreg.
20		6	Only blastulæ irreg.
21		7	Only blastulæ irreg. Few alive.

The Effect of NaOH.

The gelatinization of the egg by the addition of NaOH and other alkalines to sea water aids materially in the agglutination. When 25 drops or more of 0.5 per cent. NaOH solution was added to 200 c.c. of sea water, the gelatinization was so great, that after centrifuging, the eggs were distorted almost beyond recognition. Figs. 6 and 7, on page 75, give an inadequate idea of the degree of distortion. Less NaOH, namely, 4 to 10 drops in the same amount of sea water, gave rise to no abnormalities.

From the foregoing it is clear that within the limits specified, the various steps found necessary in the production of agglutinated eggs, either separately or all together, did not materially effect development, except for the small fragments which gave rise to atypic blastulæ and gastrulæ, already mentioned. Table V. gives a list of experiments in which numerous agglutinated and fused embryos were observed.

Table V.

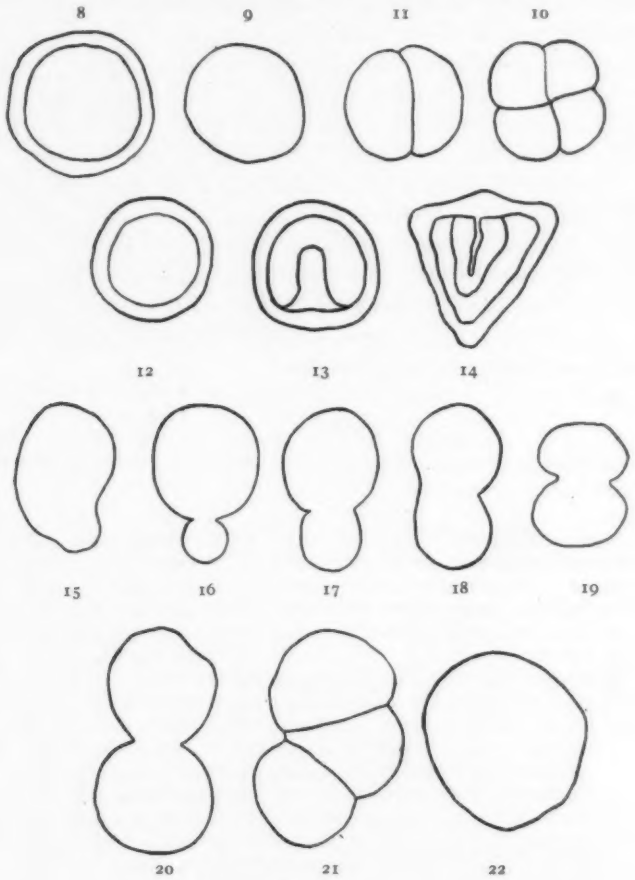
This table gives a list of experiments in which agglutinated and fused eggs were produced.

Exp. No.	Method Used.	
20.3	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	5 min.
3b2	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	7½
3b4	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	12½
25 *c5*	Fertilized, shaken, calcium-free solution, NaOH centrifuged 30×,	6
c6	Fertilized, shaken, calcium-free solution, NaOH centrifuged 30×,	8
27 *b*	Fertilized, shaken, ... NaOH centrifuged 35×,	6
c8	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	5
33 *d*	Fertilized, shaken, calcium-free solution, NaOH centrifuged 30×,	10
34 *d*	Fertilized, shaken, calcium-free solution, NaOH centrifuged 30×,	4½
37 *b3*	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	3
	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	5
	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	7
d1	Fertilized, shaken, calcium-free solution, NaOH centrifuged 60×,	2
d2	Fertilized, shaken, calcium-free solution, NaOH centrifuged 35×,	2
d3	Fertilized, shaken, calcium-free solution, NaOH centrifuged	3
d4	Fertilized, shaken, calcium-free solution, NaOH centrifuged	5
d5	Fertilized, shaken, calcium-free solution, NaOH centrifuged	7½
e2	Fertilized, shaken, calcium-free solution, NaOH centrifuged	2
38 *b3*	Fertilized, shaken, calcium-free solution, NaOH centrifuged	2
d7	Fertilized, shaken, calcium-free solution, NaOH centrifuged	5
d8	Fertilized, shaken, calcium-free solution, NaOH centrifuged	7

Etc.

THE EARLY DEVELOPMENT OF AGGLUTINATED EGGS.

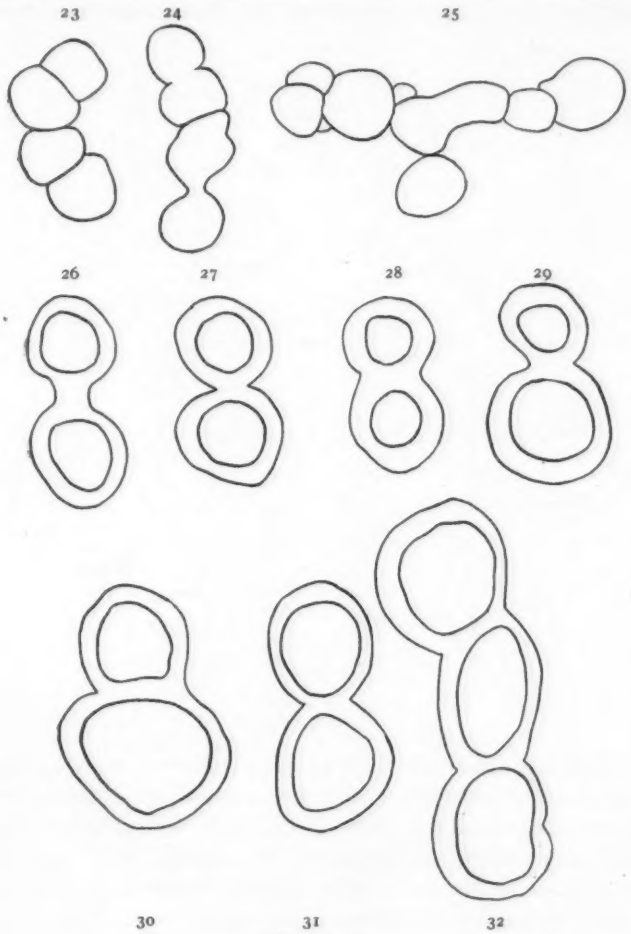
To serve as a basis of comparison, a few typical stages in the development of the normal egg are shown in Figs. 8 to 14. These



FIGS. 8–22.

were preserved, magnified and drawn with the camera lucida, to the same scale, as all subsequent ones to be described. In Fig. 8, the egg is enveloped by its fertilization membrane. In Fig. 9 the membrane is not shown. The two and four cell stages

are shown in Figs. 10 and 11. The blastula, gastrula, and young pluteus are shown in Figs. 12, 13 and 14. It need hardly be iterated that the eggs and embryos in the control cultures

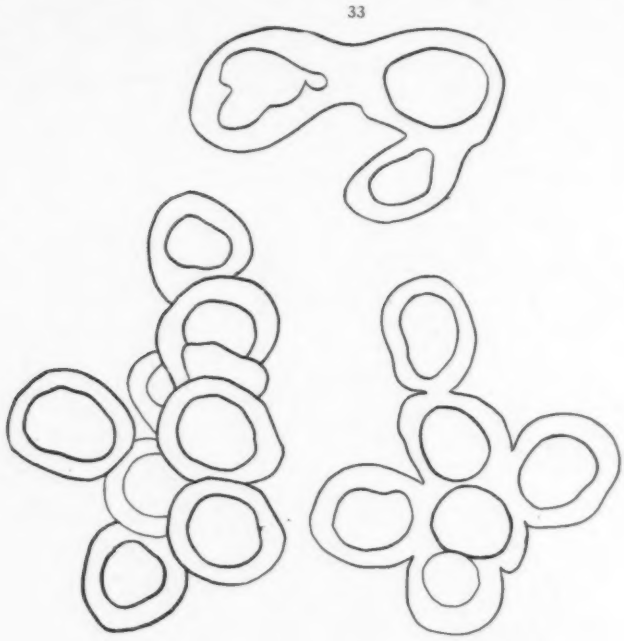


FIGS. 23–32.

varied within certain limits, as carefully worked out by Tennent ('10); the above figures represent typical or average specimens.

The treated eggs differed from the controls, in being elongated

or extra-ovate as seen in Figs. 15, 16 and 17. In Figs. 17, 18 and 19, the two parts of the egg are equal or almost equal. It is sometimes difficult to distinguish between an egg with its equal-sized extra-ovate, from one whose two blastomeres have been

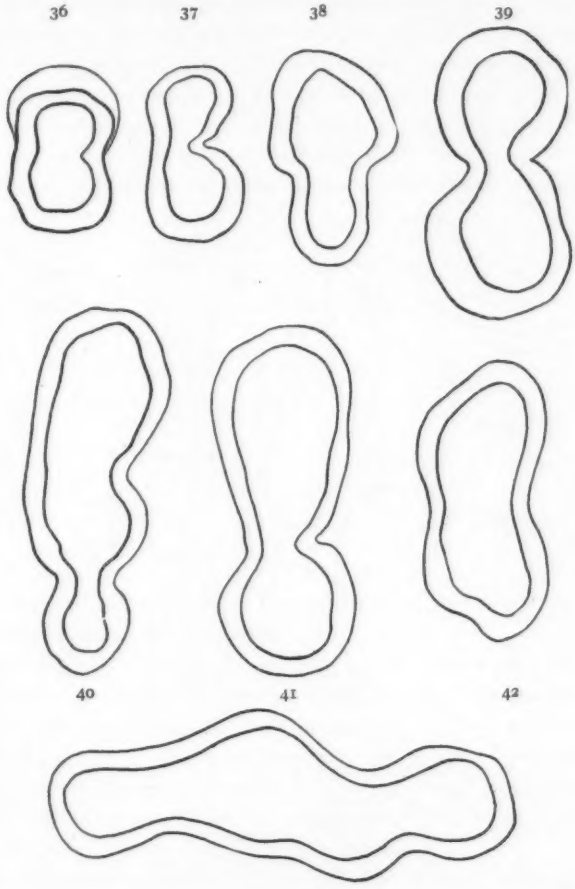


FIGS. 33–35.

partially separated. In either case the volume of the whole egg appears to be increased, sometimes suggesting an agglutination of two whole eggs, as in Figs. 18 and 19. In Fig. 20 two eggs have unmistakably agglutinated. Sometimes three eggs are compressed together as in Fig. 21, or *fused completely* as in Fig. 22.

Since the eggs or blastomeres may be agglutinated at any point of the surface and since the polarity of the egg is unaffected, the cleavage planes may occupy any angle with respect to one another, see Figs. 23, 24 and 25. Unless considerably distorted as in Figs. 6 and 7, each blastomere divided at an angle determined by the angle of agglutination or separation.

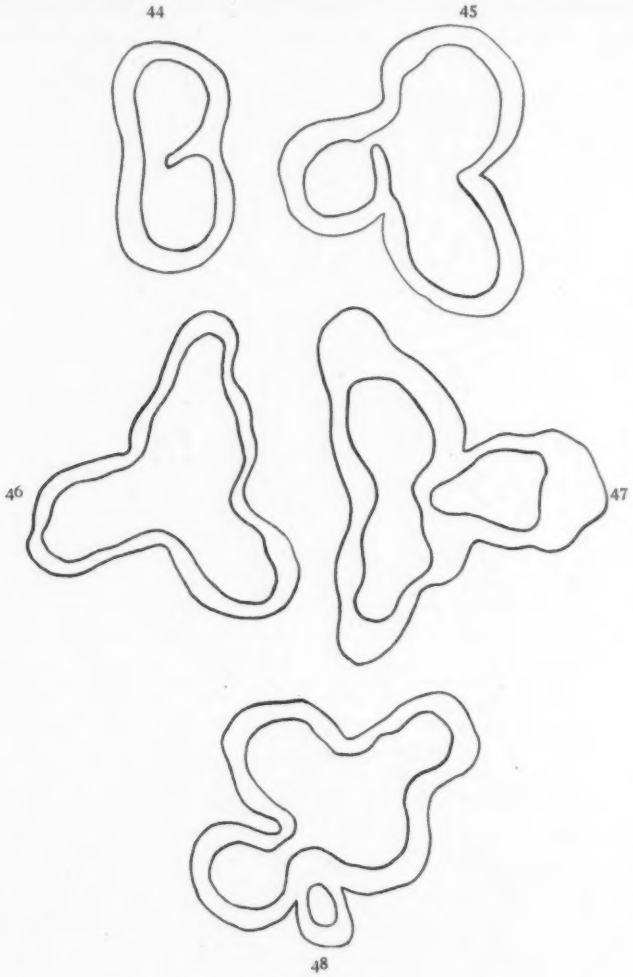
The blastulæ are particularly interesting for they make it possible to determine definitely whether the component members of a cluster are merely agglutinated or fused. Figs. 26, 27 and



FIGS. 36–43.

28 represent half blastomeres in varying degrees of approximation or separation, with their blastocœles entirely separate. Figs. 29 and 30 represent an agglutination of a half and a whole egg,

Fig. 31 an agglutination of two eggs. Three agglutinated eggs are shown in Fig. 32 and two and a half eggs in Fig. 33. Clusters



FIGS. 44–48.

of seven and nine eggs and blastomeres are shown in Figs. 34 and 35.

In a large number of instances a true fusion occurred during the blastula stage, as recognized by the continuous ectoderm and common blastocœle. In Fig. 36 a blastula is shown with a part extruded beyond the fertilization membrane. In Fig. 37 the blastula is pinched together near the middle. A whole and a half egg have been fused in the formation of the single blastula shown in Fig. 38. At least two eggs have united in Fig. 39. In Fig. 40 an egg a half egg and a quarter egg have so fused. Figs. 41, 42 and 43 represent other fusions of two or more eggs into single giant blastulæ. Figs. 44 and 45 are interesting because

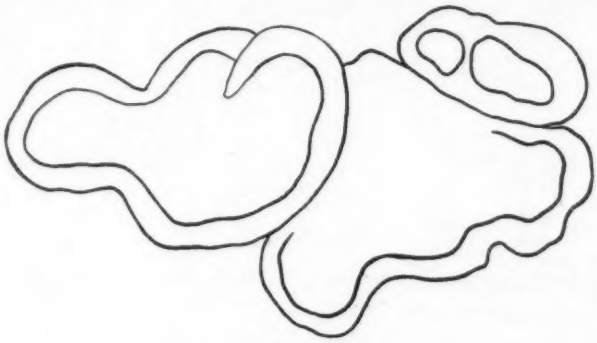


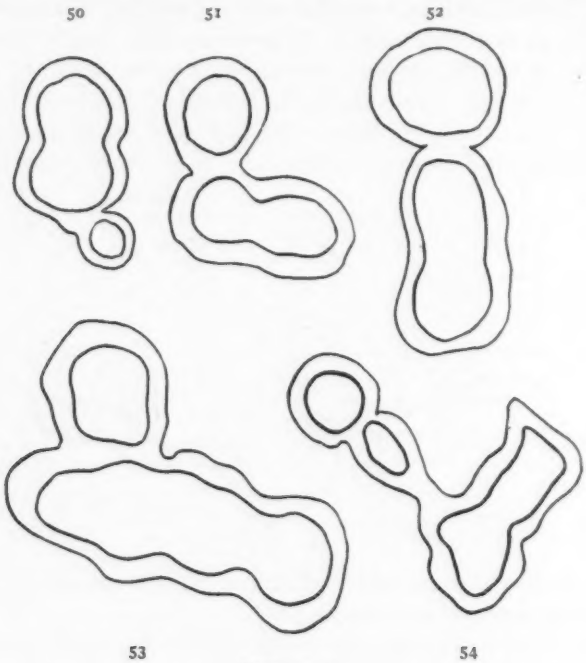
FIG. 49.

they suggest how a common blastocœle may be formed by the breaking down of the separating wall.

The clusters were not necessarily linear. Triangular groups like Figs. 45, 46 and 47 were not uncommon. Other clusters were quite irregular as in Figs. 48 and 49. A large cluster like the one shown in Fig. 49 was more frequently composed of agglutinated eggs or a complex of fused and agglutinated eggs; smaller clusters also included agglutinated and fused members shown in Figs. 50, 51, 52, 53, 54, 47 and 48.

Fused blastulæ tended to lose their individual identity by the continuity of their common layer of cells, by the disappearance of their inner separating walls and by the closer approximation of the separate blastulæ. These processes continued until a spherical or almost spherical giant blastula was produced, in which it was difficult or impossible to distinguish the component members, as in Figs. 55, 56 and 57.

The living clusters of embryos were easily distinguished from the normal ones. The latter swam with a characteristic uniformly rotary motion at or near the surface of the water. Agglutinated or fused blastulæ swam at or near the bottom swaying



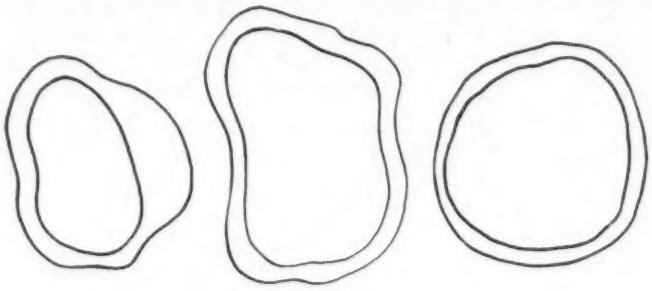
FIGS. 50–54.

irregularly and moving much more slowly. I have counted as many as fourteen full-sized blastulæ, of which Fig. 58 represents a large group of this kind. These are short lived, as already mentioned, and only the smaller clusters continue their development.

THE DEVELOPMENT OF AGGLUTINATED AND FUSED GASTRULÆ.

The differentiation of the archenteron definitely established the axes of the component members. Inasmuch as half eggs developed in precisely the same manner as whole eggs, the following statements apply to both.

The axes of the archenteron in the different gastrulæ of a cluster were found in every possible angle with respect to one another, suggesting that the polarity of the egg was unaffected by the proximity of the other. Two gastrulæ with their oral ends together, and their archentera in the same axis are shown in Fig. 59, and similar gastrulæ with their archentera almost in the same axis are shown in Fig. 60. The apex or aboral end



56 55 57

FIGS. 55–57.

of one gastrula may be attached to the side of the other and their archentera about 90° apart as in Figs. 61 and 64, or almost parallel in Fig. 62. The two gastrulæ may be attached at or near their aboral ends, Fig. 63. In Fig. 65 the oral end of one gastrula is attached to the aboral end of the second, and the archentera lie in an almost continuous line. In Fig. 66 the archentera lie in the same axis, but the gastrulæ are attached by their aboral ends. Many other examples could be cited. They clearly point to the conclusion that at this stage of development the polarity of the gastrulæ need not be changed by the proximity of other.

FUSED GASTRULÆ.

The same conclusion obtains with reference to fused gastrulæ. In Fig. 67, the blastopores are at opposite ends, namely 180° apart and the archentera have grown toward each other in the same axis. In Fig. 68, the blastopores are about 45 degrees apart and the archentera have crossed each other at this angle. Perhaps these two instances suffice as examples of this kind of independent development of the archentera. In other instances however the archentera met and fused.

In Fig. 69, the archentera have approximated but not yet fused into one continuous gut. In Fig. 70 the two have unmistakably united into a single continuous gut with no trace of blastopores.

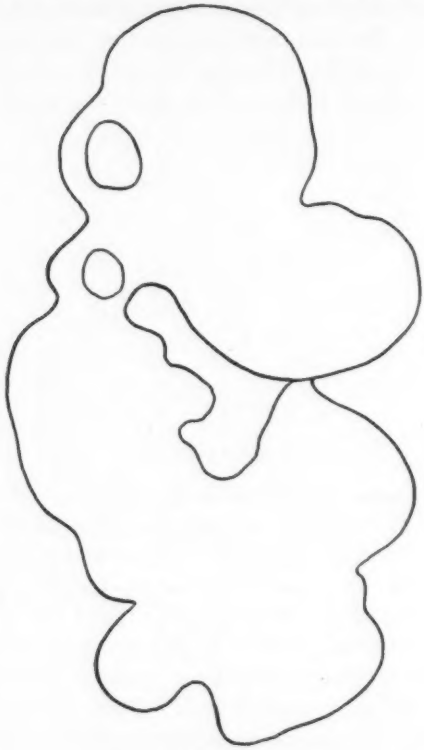
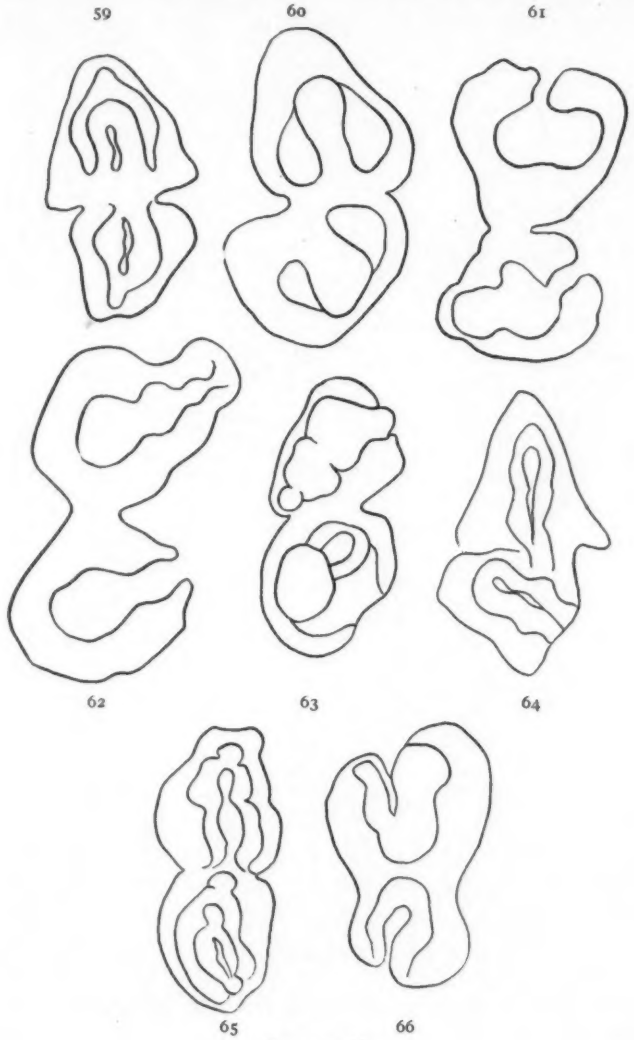


FIG. 58.

A similar gastrula drawn from life and drawn at greater magnification than the rest is shown in Fig. 71. In a cluster of four gastrulæ two have separate guts, two have united ones, Fig. 72.

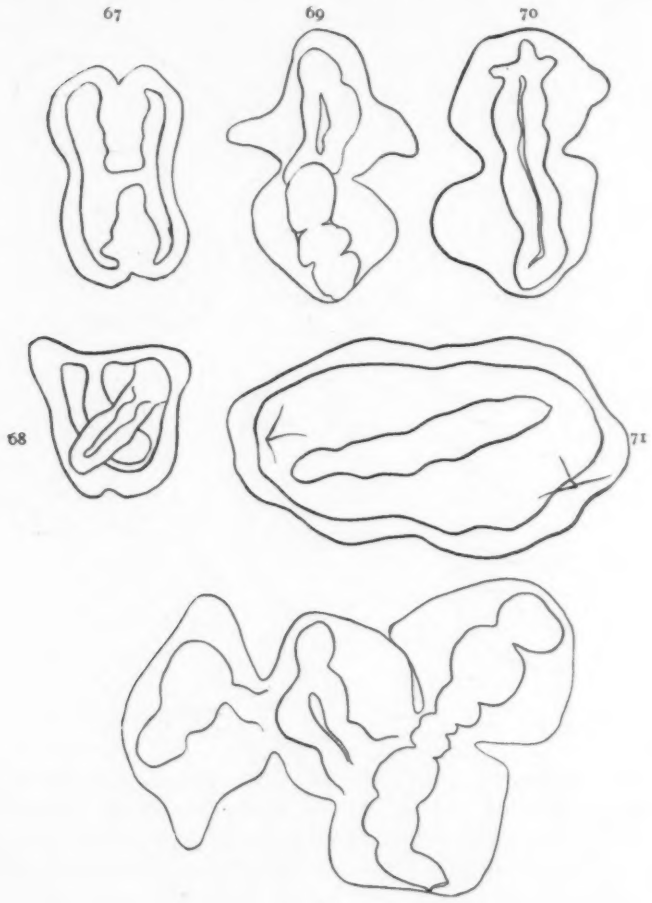
Instead of fusing end to end, the archentera may unite near their middle as in Fig. 73, or the blastopores with or without parts of the archentera may be united as in Figs. 74, 75 and 76. If one were to suppose the fusion of two archentera side to side, as suggested by the preceding figure, one would anticipate the single giant archenteron shown in Figs. 77 and 78.

There were in the different cultures two other kinds of fusion that deserve brief mention. In the one, a gastrula developed in only one of the pair of eggs or blastomeres as shown in Figs.



FIGS. 59–66.

79 and 80. The archenteron in such fusions may grow beyond the boundaries of the blastomere or egg into the blastocœle of its neighbor as seen in Fig. 81., In the second group the archentera

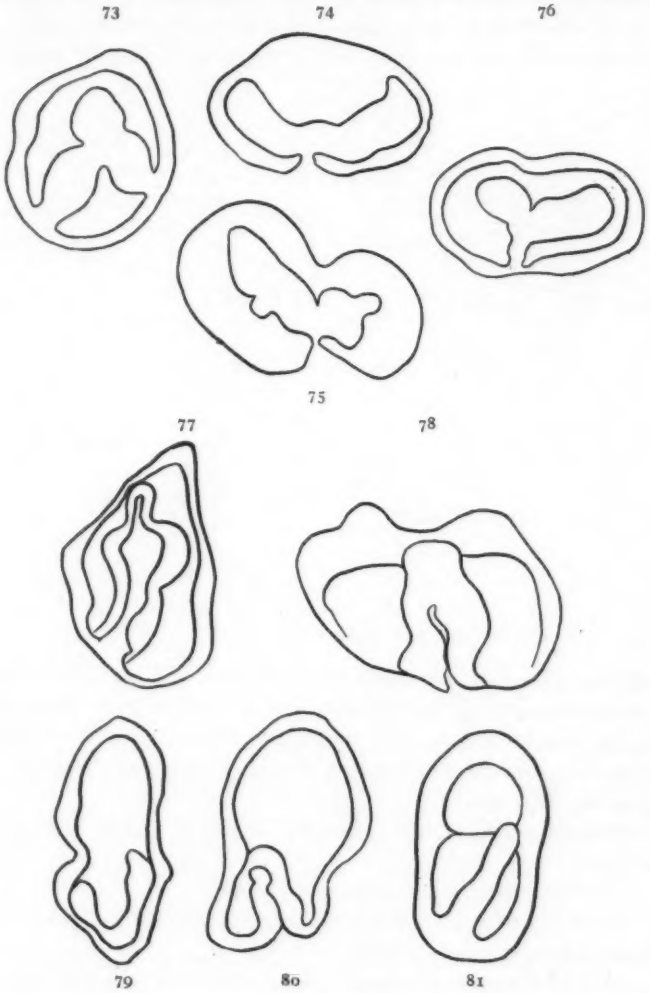


FIGS. 66–72.

are usually atypic either detached as in Fig. 82 or independent, Figs. 83 and 84.

THE DEVELOPMENT OF AGGLUTINATED AND FUSED PLUTEI.

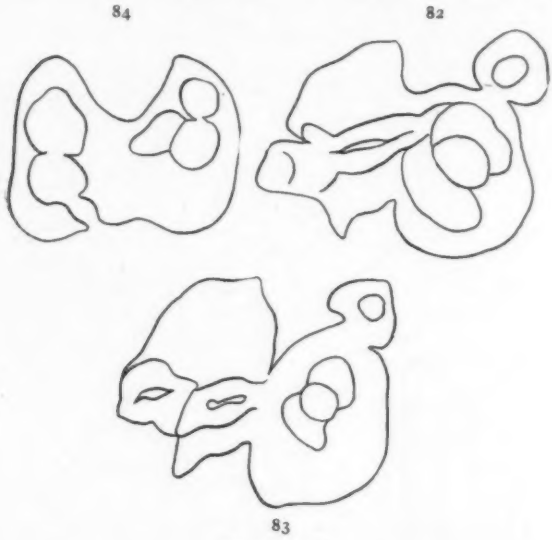
It will be recalled that the agglutinated embryos tended to separate and that this tendency increased during development. When the pluteus stage was reached, very few remained aggluti-



FIGS. 73–81.

nated, and these were in nearly every instance, clusters of two. The separation of the embryos of *Arbacia* occurred in exactly the same manner as described by Driesch.

These permanently agglutinated plutei may be attached to one another at any angle as in Figs. 85, 86 and 87. In Fig. 85 the plutei are attached by their oral ends; in Fig. 86, the oral end of one is attached to the side of the other, and in Fig. 87 a group



FIGS. 82–84.

of five plutei are agglutinated at various angles. As in the previous stages of development one finds here also, that the agglutinated partners may develop at different rates, the one a pluteus, the other a blastula as Fig. 88, or a pluteus and a gastrula, Fig. 89.

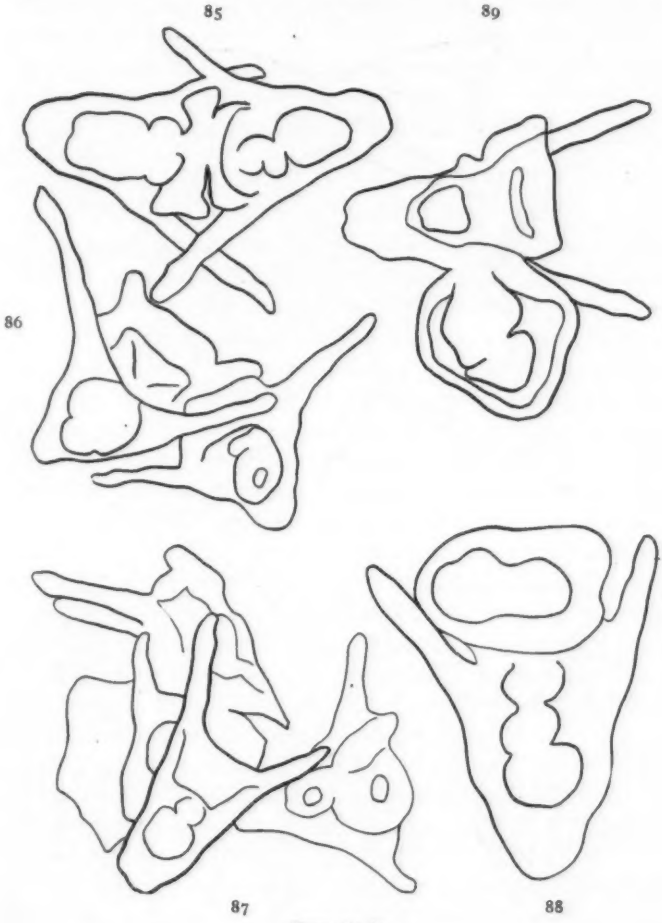
Driesch described the following types of plutei from his cultures:

(*a*) True twins, *i. e.*, agglutinated plutei.

(*b*) Twins with a common blastocœle, *i. e.*, body wall partially fused, internal organs double.

(*c*) Twins with a reciprocal influence on growth, a true fusion with an enlargement of the body.

(*d*) Fusion with partially double archenteron.
(*e*) True fusion with a single set of organs.
(*f*) Single body with a second parasitic archenteron.
Some of these types he obtained from *Echinus microtuber-*

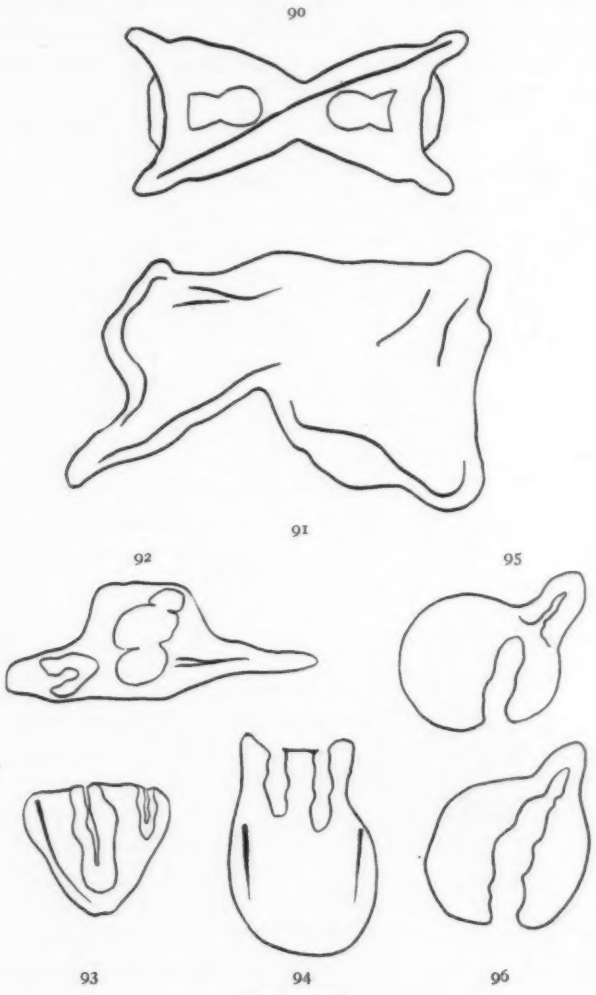


FIGS. 85–89.

culatus only, types *a*, *b*, *c*, *d* and *e*; others from *Sphærichinus granularis*, types *e* and *f*; and some from both of these echinoderms, type *e*.

Since Driesch has so clearly and accurately described these types, there is no need to enter into detail except to mention that with *Arbacia punctulata*, I obtained all of them, namely:

Type *a*, or true twins, Figs. 85, 86 and 87.



FIGS. 90–96.

Type *b*, pair of plutei with a common blastocœle, Fig. 90, drawn from life at greater magnification.

Type *c*, fusion of two, but with double set of organs, very few examples, Figs. 94 and 82.

Type *d*, fusion of two with partially double archentera, Figs. 73, 74 and 75.

Type *e*, complete fusion with but a single set of organs, the pluteus of Figs. 88 and 78.

Type *f*, single body with a second parasitic archenteron, Figs. 84, 92 and 93.

The Development of Individual Clusters.

In an effort to follow the developmental and regulative processes, during agglutination and fusion, each cluster was isolated and examined at periodic intervals. The very large groups separated into their component members, as already pointed out, each developing into a normal larva or the inner ones disintegrated bringing about the disintegration of the entire cluster. The very small groups usually remained intact. Sketches and memoranda made during the development of these small clusters clearly showed that the types of agglutinated and fused eggs, embryos and larvæ described in the previous section, were not the artificial results of preservation, but represented various serial steps in the development and regulation of such clusters. To describe these isolated groups would be to repeat the descriptions of preserved material given in the preceding section.

This statement applies with equal force to fused clusters, though not all types were observed in the isolated clusters. The following are some of the true fusions observed:

Two eggs fused into a single body with two independent archentera equal in size, Fig. 94,[1] or unequal, Fig. 93.

Two eggs fused into one body with two archentera attached end to end.

Three eggs fused into one body with three independent archentera.

Two fused eggs and one egg fused to a blastomere developed independent archentera, Fig. 95, which subsequently came in contact and fused into one very long archenteron, Fig. 94.

[1] Figs. 93 to 95 are drawn from hand sketches.

Two eggs fused into one pluteus with independent archentera, one very much larger than the other.

Two eggs fused completely into one pluteus body with one set of organs.

Other clusters showed distinct retrogressive and involutional changes of which the following are a few examples:

Four blastulæ were agglutinated in a row. The end ones enlarged, the inner ones became small. One of these small blastulæ developed an archenteron which later shrank and disappeared completely. Certain other changes occurred which altered the character of the group so that ultimately three minute blastulæ were crowded together at one point on the periphery of a large blastula.

Two large fused blastulæ were attached to a third blastula very much smaller. The three fused into one body with three independent archentera. The next day the small archenteron disappeared, skeletal rods differentiated on one side of the body constituting a fusion of a pluteus and a gastrula. Two days later the parts had fused more completely, into a single body with a single normal-sized archenteron and a single skeleton.

Two eggs developed into fused gastrulæ and later into fused plutei, attached by their oral surfaces. One of the plutei decreased in size while the other increased correspondingly, the total linear dimensions remaining constant. The smaller pluteus ultimately became less than one quarter its original size.

Summary.

Prior to this work, no one had succeeded in fusing at will the eggs of any animal found on this side of the Atlantic. By a modification of the Herbst-Driesch method, described in the text, it was possible to agglutinate and to fuse relatively large numbers, namely 10 to 40 per cent. of the sea urchin *Arbacia punctulata*.

Such clusters whether studied in mass cultures or in isolated groups, developed into all the types of larvæ described by Driesch for *Echinus microtuberculatus* and *Sphærechinus granularis*, namely:

(*a*) True twins.

(*b*) Twins with a common blastocœle.

(*c*) Twins with a reciprocal influence on their growth.

(*d*) Fusion with partially double archentera.

(*e*) True fusion with a single set of organs.

(*f*) Single body with a second parasitic archenteron.

Since little attention has been given to the early development of such agglutinations and fusions, the text emphasizes the earlier stages summarized as follows:

Clusters of 2 to 20 eggs and blastomeres were successfully agglutinated. The large clusters nearly always disappeared either by the separation of the outer members or by the death and disintegration of the inner ones. Small clusters of 2, 3 or 4 eggs or blastomeres survived and either remained agglutinated or were fused.

The eggs were agglutinated either at the egg stage or during the formation of the blastula. In clusters which remained agglutinated and did not fuse, the members developed independently, *i. e.*, the polarity was not affected by the proximity of the other, and the rate of development was not necessarily different in the individual members of a cluster.

Fusion occurred infrequently at the egg stage, but more commonly took place during the blastula or later stages. Such fusion involved either the body wall, one or more of the internal organs or all of these.

Fusion was frequently determined by the degree of compression of the component eggs or blastulæ, as well as the position and angle of attachment.

Embryos fused end to end frequently developed a single archenteron, about twice the normal size, either with or without blastopores.

Embryos fused with their axes 90 degrees apart, frequently fused in such a manner that their archentera united at the point of contact.

Embryos fused with their axes parallel and close together, frequently fused in such a manner that the two archentera fused into one, along their whole length.

The study of individual clusters served to show that these types of agglutination and fusion represented regulative and

sometimes involutional stages in the development of these clusters.

The evidence supports the view that not only may an egg give rise to several perfect larvæ, but that several eggs may be united so as to constitute a single larva, with or without traces of its duplicate nature.

In conclusion I wish to thank Prof. F. R. Lillie for placing the facilities of the Marine Biological Laboratory at Woods Hole, Mass., at my disposal.

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ON THE HABITS OF THE CRUSTACEANS FOUND IN CHÆTOPTERUS TUBES AT WOODS HOLE, MASSACHUSETTS.

A. S. PEARSE,

ZOÖLOGY DEPARTMENT, UNIVERSITY OF WISCONSIN.

Among the numerous species of crustaceans which live as commensals, three interesting representatives occur in the leathery tubes of the worm *Chætopterus variopedatus* Renier et Claparède, at Woods Hole. The tubes of this annelid are U-shaped and taper toward the round opening at either end (Fig. 7). The tips of the tube may be seen protruding above the mud in open shallow water where eel-grass grows. Enders ('05, '09) has published two papers describing the habits of *Chætopterus* and its commensals as he observed them at Beaufort, North Carolina. Van Beneden ('76, p. 20) also mentions a worm, which is doubtless the same, as associated with crabs on the coast of Brazil. While occupying a room in the Marine Biological Laboratory during the past summer I was able to verify Ender's work and to add some new observations. My thanks are due to the staff of the laboratory for their courtesy, particularly to Mr. George Gray.

Enders ('05) took four species of crustaceans (*Polyonyx macrocheles* (Gibbes), *Pinnixa chætopterana* Stimpson, *Pinnotheres maculatus* Say, *Menippe* sp.?) from tubes at Beaufort. All of these crustaceans except *Menippe* were taken at Woods Hole. Usually a male and a female of the same species were found together in each tube, but several solitary individuals were captured, and once two *Pinnixæ* of each sex were found in a single tube. The results of the collections at the two localities are compared in Table I. The table shows that *Polyonyx* is the most abundant commensal at Beaufort, whereas *Pinnixa* is the most frequent at Woods Hole. Mr. George Gray, who has collected *Chætopterus* for several years at the latter place, affirms that he had never observed *Polyonyx* until about 1909, and that

SHOWING THE NUMBER OF ANIMALS FOUND IN *Chætopterus* TUBES.

Locality.	*Polyonyx macrocheles.*		*Pinnixa chætopterana.*		*Pinnotheres maculatus.*		*Menippe* sp.?	*Nereis* sp.?	*Pinnixa* bearing *Hippasteria elongata.*		Number of *Chætopterus* Tubes, with or without Commensals.		
	♂	♀	♂	♀	♂	♀			♂	♀	Present.	Lacking.	Total.
Enders at Beaufort, N. C.	71	73	15	15	2	2	1	2	–	–	89	10	99
At Woods Hole	14	19	57	61	2	0	0	–	4	7	80	9	89

it has been increasing in numbers since then. The size of the crustaceans collected at Woods Hole was about the same as those measured by Enders ('05) at Beaufort. The maximum width of the carapace recorded for *Polyonyx* was: female, 12.5 mm.; male, 9 mm.; *Pinnixa:* female, 12.5 mm.; male, 13 mm. The proportion of tubes containing commensals was about the same at the two places.

Pinnotheres was not studied on account of its infrequent occurrence, but the behavior of *Pinnixa* and *Polyonyx* was observed in some detail. Both the latter crustaceans are thigmotropic. When placed in a dish they usually stayed close in the angles at the edge, or crawled under any objects that were present. *Pinnixæ* often clung to each other or piled up in groups. If supplied with glass tubes of suitable size, they crawled into them and there remained indefinitely.

In order to test the behavior of the commensals toward *Chætopterus* tubes an artificial tube was made in which their movements could be observed. A glass tube was bent in the form of a U and the tip of a *Chætopterus* tube slipped over each end where it was tied securely. This composi te tubewas placed upright in a rectangular glass jar filled with sand so that the glass tube could be observed through one side of the jar, though only the leathery tube tips projected into the sea water above the sand. The crustaceans were placed on the surface of the sand and their behavior observed. *Pinnixæ* usually walked to one side of the jar where they burrowed into the sand and remained for several minutes; the *Polyonyces* moved quickly to the side

of the jar and crouched on the sand. None of the crustaceans moved at once to the projecting tube tips, and sometimes they even came in contact with a tube without reacting. After a time, however, they would begin slowly exploring the surface of the sand, and, if they then came in contact with a tube, usually entered at once, whether a *Chætopterus* was inside or not. Apparently they found the tubes by accident.

In regard to the ability of the commensals to leave the tube Van Beneden ('76, p. 20) says, "On the coast of Brazil, my son found two couples of crabs in the tube of a very long annelid, narrow at the ends, and wide in the middle. The tube was too small at the end to allow them to escape. These crustaceans had, no doubt, penetrated thither before they had attained their full size. Enders ('05, p. 39) also believes that, "Once in, the crabs remain there and are later prevented through their own growth from escaping. . . . When a worm in a tube dies the crabs in the same tube die as a result of the failure of food and properly aerated water." Some tubes may be too small to allow the commensals to pass in and out, but there can be no doubt that they enter and emerge again in some cases, as the two following experiments show.

Experiment 1.—On July 19 three *Pinnixæ*, two males and a female, were placed on the sand in the jar containing the tube previously described, which contained a living *Chætopterus*. Next morning all had entered the tube; the *Chætopterus* was dead. At 2.00 p. m, two of the crabs had come out on the surface of the sand, but one still remained in the tube.

Experiment 2.—After the last experiment the tube was removed, thoroughly cleaned, and replaced in the jar with fresh sand and sea water, but no *Chætopterus* was placed in it. On July 20, at 5.00 p. m., the two largest *Pinnixæ* obtainable (male, 13 mm. wide; female, 12.5 mm.) were placed on the sand; at 9.00 a. m. the next morning the male was in the tube, and at 12.30 p. m. the female had entered. On July 22, at 3.00 p. m., a male (9 mm.) and a female (12.5 mm.) *Polyonyx* were also placed in the jar. The male entered at 3.21 and the female at 3.40 p. m. All four crabs remained within the tube until July 24 at 4.45 p. m., when I observed that the water had become foul;

the female *Polyonyx* had left the tube and was on the surface of the sand. The other three crustaceans stayed within until July 27, at about 8.00 p. m., when the male *Polymyx* emerged. The water in the jar at that time was murky, but did not have a very bad odor. On the morning of July 29 the water had some odor; the *Polyonyces* were dead on top of the sand and the *Pinnixæ* had left the tube but were alive and active.

The crabs in experiment 2 were the largest obtainable and the apertures at the ends of the tube tips were of medium size. They seemed to have little difficulty in passing in or out, though one would not think so at first glance. *Pinnixa* walked in sideways; *Polyonyx* extended its chelæ and one of them preceded the body, the other followed.

Locomotion outside the tubes was first studied in a large flat dish containing sea water. *Pinnixa* did not use its last leg when walking forward, but when going sideways used all the walking

FIG. 1. Diagrams to represent the method of locomotion used by *Polyonyx* when resting on its setal fringe with its body against some object.

legs, except the chelipeds, which were hugged close against the body; it was never seen to move backward. *Polyonyx* usually moved backward when in the open and sideways along the sides of the dish. In the latter situation it always faced toward the center of the dish, or, if in haste, stood with its ventral surface against the side of the dish and walked along on the tips of its chelipeds and the bristles along their outer edges (Fig. 1). If very much hurried an animal sometimes turned on its back and flapped its abdomen, thus swimming slowly. When placed in a vertical glass tube filled with sea water *Polyonyx* kept from falling by bracing the fringe of bristles on the chelipeds and the second and third walking legs forward; the fourth leg serving as a prop behind (Fig. 7). The small fifth leg was not used. Locomotion was always sideways. In a similar situation *Pinnixa* braced with all its walking legs; the first and second were extended forward, the third below, and the fourth and fifth

backward. The largest and strongest leg, the fourth, served as a sort of hook to grasp any inequality in the wall of the tube, and the smaller fifth leg was often used in much the same way. In a horizontal tube *Pinnixa* moved sideways or stood in a position like that when on a flat surface; *Polyonyx* usually rested on the fringe of bristles along the chelipeds with its head down. *Pinnixa's* most effective locomotor organs are the fourth walking legs; *Polyonyx* uses the chelipeds most.

Pinnixa is an expert burrower, but *Polyonyx* has little ability in that line. When placed in a bowl containing sand and sea water a *Pinnixa* would scratch for a moment with all the walking

FIG. 2. *Polyonyx* and *Pinnixa* in Syracuse watch glasses. × 4/7. *a, Polyonyx*—male at left, female at right. *b, Pinnixa;* the lower individual is covered by the bryozoan *Hippuraria elongata* Osborn.

legs except the chelipeds, then stop moving the legs on one side and quickly burrow sideways by scraping the sand away with those of the other. Usually one would bury itself completely in a minute or two. If *Polyonyx* was placed in the same bowl it did not burrow, in fact was never seen to burrow, though occasionally an individual would wiggle itself down into the sand a little.

Respiration is a matter of importance to an animal that lives in a worm tube imbedded in a muddy flat. Yet *Pinnixa* apparently takes no special precautions to secure well-aerated water and is able to endure a much greater degree of foulness than *Polyonyx*. Its respiratory currents are feeble and incon-

stant both as to force and direction. In *Polyonyx*, however, such currents are strong and well adapted to life in tubes. When an individual is placed in a dish of water, a current will soon be seen to issue from beneath the anterior end. Sometimes it passes straight forward, but more often moves to the right or left,—as it would have to do if the crustacean were in a tube.

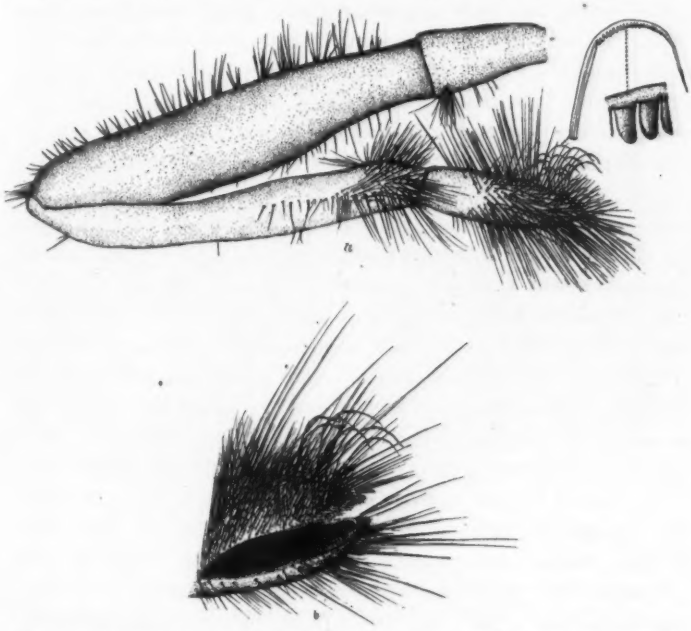

FIG. 3. Cleaning leg of *Polyonyx*. *a*, entire leg with details of pectinate setæ; *b*, chelate tip of leg.

The direction of the respiratory current in a particular individual may change frequently in a dish, but in a tube it is usually constant for hours at a time. Furthermore, several individuals in a tube together will adopt the same direction so that the water passes in one end of the tube and out the other. *Polyonyx's* antennules assume a peculiar position in relation to the respiratory current. Both are bent against the current and take such a position that the smooth ramus (Fig. 5*a*) meets it first; and if the current is changed, the position of the antennules is always

altered at once. These appendages are waved quickly at regular intervals while extended.

Polyonyx is very cleanly. The last leg is not used for locomotion, but is peculiarly modified, as in many anomurnas, to form a cleaning organ (Figs. 3, 7). The tip is chelate and is provided with peculiar pectinate and plumose setæ. The crustaceans use these appendages with great expertness; they can reach all parts of the body with them, even the interior of the gill chambers, and can frequently be seen industriously currying themselves. As a result, the body is always as smooth and clean as new porcelain. *Pinnixa* apparently takes little care for its cleanliness. Except for the mouth parts, antennæ and eye stalks, the body is usually dirty and over-grown with various organisms. A bryozoan completely covered the exposed parts of several of the individuals captured (Fig. 2*b*), a *Vorticella*-like protozoan was attached to others, and one crab was observed that carried a small clam, *Mytilus edulis* (Linnæus), attached to its last leg by byssal threads. Concerning the bryozoan Osborn ('12) says: "*Hippuraria elongata* is also a commensal living in the branchial chambers of the blue and spider crabs and on the carapace of *Pinnixa* living in the tubes of *Chætopterus*." This species is apparently not found except in association with crabs.

In most of the particulars described the two crustacean tenants in *Chætopterus* tubes showed striking dissimilarity, but their feeding habits were very much alike. Both *Polyonyx* and *Pinnixa* obtain their food by "net-fishing" (Calman, '11, p. 115) like barnacles. The "nets" in both are formed by the endopods of the third maxilliped which are well supplied with plumose setæ. A *Polyonyx* by spreading his nets (Fig. 4) can strain most of the water passing through the tube where he lives. They are extended laterally and swept together below the body. Captured food is carried against the ventral side of the body where it is scraped from the setæ of the net by the mouth-parts. *Pinnixa* feeds in a similar manner but the nets are raised above the head and swept forward and downward against the mouth. Various small organisms are captured. Eight fresh *Pinnixa* stomachs were examined on August 5 and found to contain (in order of abundance): pieces of algal filaments, diatoms, a flagellate

(*Exuvella*), fine silt, and other unidentifiable particles. Five *Polyonyx* stomachs examined August 9 contained: diatoms, silt, algal filaments, and spores or cysts. The commensals apparently feed on such organic matter as can be strained from the water passing through the worm tubes in which they live.

Enders ('05) points out that a prolonged breeding period characterizes *Polyonyx* and *Pinnixa*, and he believes this condition is due to the protection afforded by the worm tubes.

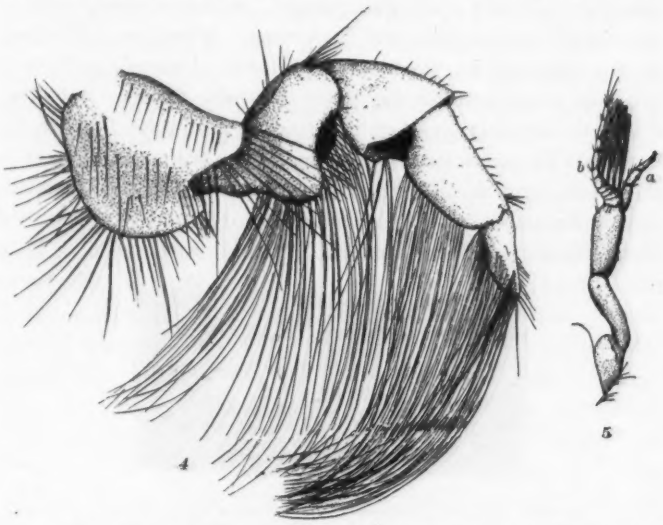

FIG. 4. The endopod of the third maxilliped of *Polyonyx*—"the fishing net." The long setæ on the four distal segments are plumose (not shown in the figure).

FIG. 5. First antenna (antennule) of *Polyonyx*.

Every female he took at Beaufort, from June 21 to October 25, bore eggs or had recently shed them. Every female taken by the writer at Woods Hole (July 18 to August 9) bore eggs or young. Four females shed their zoëa in the dishes where they were kept in the laboratory. The bluish eggs of *Pinnixa* are well protected by the broad abdomen which folds tight against the body and completely covers them. Those of *Polyonyx* are bright red; they project so that they are not concealed by the abdomen and appear as a bright mass between the body and the chelipeds when the crustacean is viewed from above (Fig. 2*a*).

The light reactions of the commensals were tested. Animals were placed in a flat dish containing sea water. This dish was barely enclosed by a box two feet long and sixteen inches wide. The box was painted black on the inside and light was admitted through an aperture two inches high across the lower side of one end. When a *Pinnixa* or a *Polyonyx* was placed in the box before a window it usually went toward the light and tried to get through the glass for a time. It soon began to wander about the dish, however, and after twenty-four hours spent most of the time in the darkest end of the box. When ten individuals of the same species were put in the dish simultaneously their behavior was essentially the same. Twenty *Pinnixæ* and seven *Polyonyx* were left together in the box several days. When the lamp was lighted before the opening of the box at night most of the crustaceans went toward it and tried to get through the end of the glass dish, but after an hour they became scattered about the dish without particular reference to the light. Mast

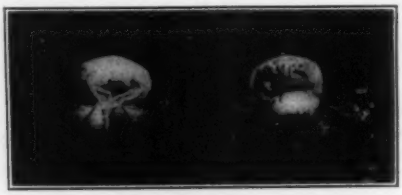

FIG. 6. *Pinnixa* undergoing ecdysis. The left-hand figure shows a ventral, the right-hand a dorsal view.

('11, p. 284) mentions several animals which, though usually negatively phototropic, may become positive for a time after mechanical or other stimulation. Apparently steady light is not, as a rule, an important factor in the daily life of *Pinnixa* and *Polyonyx*, but if confined under unnatural conditions they may go toward a light. Such reactions might enable them to escape from confinement. Both *Pinnixa* and *Polyonyx* responded readily to a decrease in light intensity. The former was very active when placed in a glass dish and kept its legs continually in motion; but if an object was passed between it and the window it became motionless at once. Though *Polyonyx* was more

sluggish it showed similar sensitiveness to shadows. Such reactions would of course help to protect these animals from predaceous enemies.

On July 24 one of the *Pinnixæ* in the "dark box" liberated a number of zoëa larvæ which swam persistently against the glass toward the light for an entire day; they were then removed.

Pinnixæ underwent ecdysis in the dishes in which they were kept; one shed on July 24 and two on August 5 (Fig. 6). They were apparently much hardier than *Polyonyx* and did not throw off their legs readily nor die if the water became foul. *Polyonyx*, like all its near relatives (Calman, '11, p. 114), readily practices autotomy, and quickly succumbs to unfavorable conditions. Its extreme cleanliness has already been mentioned.

General Discussion.

Perhaps the chief point of interest in these dissimilar crustaceans which have come to be associated with *Chætopterus* so closely that they are rarely found elsewhere lies in the similarities in physiology and structure which have enabled them to take up such a peculiar mode of life. The similarities ought to point to essential or fundamental characteristics from an ecological point of view, and the unlike features should be of less importance.

Let us first examine the differences between *Polyonyx* and *Pinnixa*. As to relationship, both are decapods, but the former belongs to the family Porcellanidæ (tribe Galatheidea, section Anomura) and is therefore not a crab despite its general appearance; the latter is a true crab of the family Pinnotheridæ (tribe Brachygnatha, section Brachyura). The Porcellanidæ use only three pairs of legs for walking, the first forming the chelæ and the last being very small and carried folded up at the sides of the body or even within the gill chambers. They are mostly found under stones along the sea shore and among corals. The family Pinnotheridæ contains many crabs which live as commensals in molluscs, worms, sea-urchins, and other animals. Their shells are often softened or membranaceous, and their eyes are very small; both these characteristics have been supposed to have arisen as a result of commensal life (Stebbing, '93, p. 100); the fifth legs are much shorter than the fourth but are used for

walking. *Pinnixa* never walks backwards, while *Polyonyx* never walks forwards; but both move sideways.

Pinnixa is very hardy and can stand foul water, as well as the indiscriminate growth of organisms on its carapace, and does not have a strong respiratory current. *Polyonyx* is not hardy, but takes every precaution to protect itself. It has a special cleaning appendage; when at rest it stands high on its setal fringe above the dirt that may collect under it; it never burrows; it has a very strong respiratory current which is deflected laterally so as to clear its abode. Apparently *Pinnixa* can endure great hardship through its great resistance, and *Polyonyx* has a number of adaptations to protect itself from contamination. The former could live almost anywhere, the latter is adapted to life in *Chætopterus* tubes or other protected situations.

The similarities between these commensals are as follows: Both are, like most crustaceans, strongly thigmotropic, and creep into crevices or tubes; they become quiet when a shadow passes over them; they feed by "net casting" after the manner of barnacles; both have a very long breeding season, producing one brood after another; and both have the last leg shortened. Their thigmotropism would easily account for their entering *Chætopterus* tubes and their feeding habits are admirably suited for the capture of food in such a situation. The quick cessation of motion when stimulated by a decrease in light might protect them from enemies when out of their tube. Enders ('05) believes that the long breeding period is an adaptation that has arisen as a result of the protected life in the worm tubes.

Shelford ('11, p. 603) says: "An animal should be associated: first, with breeding conditions; second, with the feeding conditions; third, with the conditions affecting shelter." In the present case, in fact in the case of most crustaceans, external conditions are of consequence in breeding only in limiting parents to a general region where the larva may carry on its later development, for the eggs are carried by the parent for a longer or shorter time. Some crustaceans (*Birgus*) have probably adapted their reproduction to suit a particular environment. The feeding habits of *Polyonyces* are such that they might exist anywhere in shallow water. It is apparently for protection that they have

taken up life in *Chætopterus* tubes. While this in no way disproves Shelford's statement as a general proposition, the three factors would, in the present case, come in the following order; the most important first: (1) protection, (2) food, (3) breeding. Of course, if suitable conditions for breeding were absent the crustaceans would become extinct; but given the racial habit of carrying eggs and the abundance of pelagic microörganisms in littoral waters for food, reproduction takes care of itself, is fostered, in fact, and its products are unusually abundant.

Calman ('11, p. 217), in speaking of the association of Pinnotheres with molluscs, says, "The case is, indeed, an example of the difficulty of defining these two terms (commensal, parasite). At all events the Pinnotherid crabs show one of the characteristics of parasites in being to some extent degenerate in their structure. The carapace and the rest of the exoskeleton, no longer needed for protection, have become soft and membranous, and the eyes and antennules, the chief organs of sense, are very minute. As in many parasites, also, the eggs are very numerous, and the abdomen is very broad and deeply hollowed out for their reception." To this list I may also add that *Pinnixa's* fourth and fifth legs are used as hook-like claws for holding on, like the organs of fixation in many parasites.

There is no question but that the two commensal crustaceans discussed gain from their association with *Chætopterus*, but it is doubtful if the worm is benefited.

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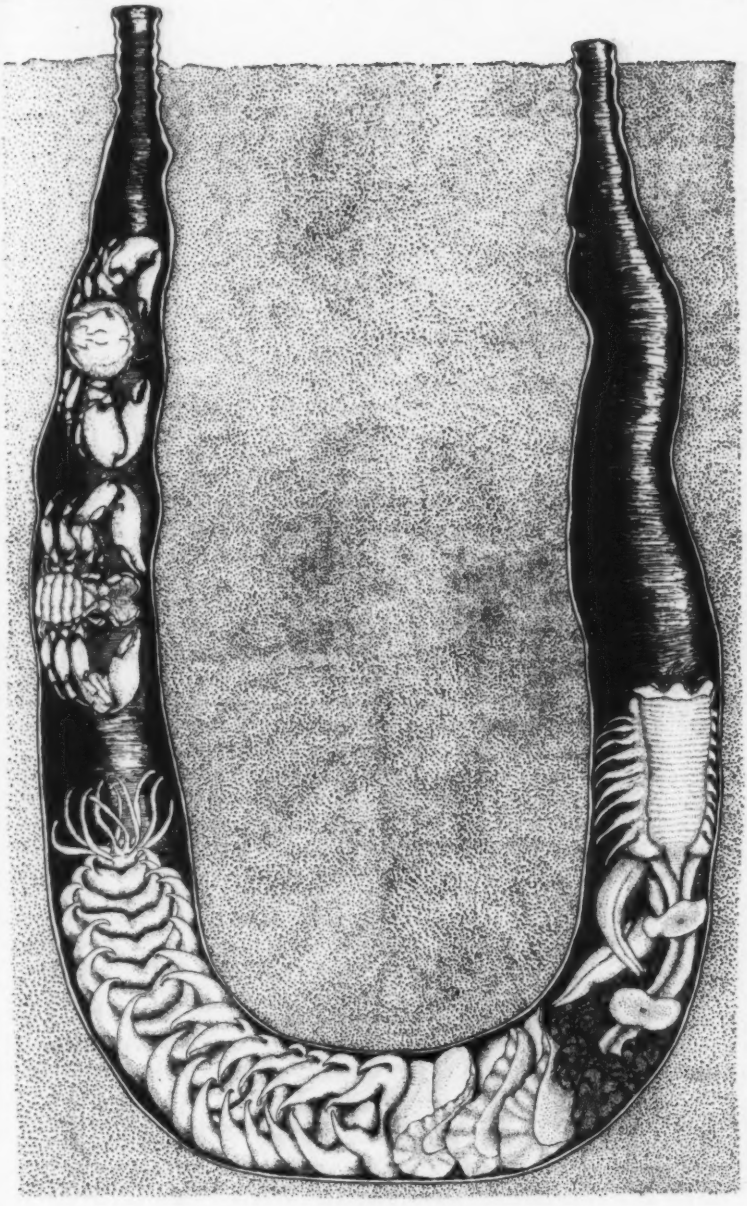
Explanation of Plate I.

Fig. 7. Section of a *Chætopterus* tube in the sand. It contains a worm in the lower part. On the left a male (above) and a female *Polyonyx* are clinging to the wall of the tube. The male is cleaning his back with the comb on the tip of his last leg; the female rests with her ventral side toward the observer. Drawn from life by Miss Barbara Bradley.



SEX RATIOS IN DROSOPHILA AMPELOPHILA.

ELIZABETH RAWLS,
COLUMBIA UNIVERSITY.

As a rule there is an excess of females amongst the fruit flies that first hatch. It seemed that this must be due to the more rapid development of the females since the total output gives approximately an equal number of males and females. It was possible, although improbable, that more females are actually *produced* at first and more males later. In order to test this possibility five pairs of wild flies from stock that had been a year in confinement were placed in bottles and after two days removed to new bottles. Then after two days each pair was removed to a third bottle, etc., as long as the pair lived. Counts were made of all the flies produced in each bottle. The results showed that on an average about equal numbers of males and females came from each batch. This result occurred in the case of three of the five pairs of flies studied, as the following figures will show:

TABLE I.

No. of Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	375	331	1 : 1
No. 2	309	281	1 : 1
No. 3	324	335	1 : 1

The fourth pair began very early to show a somewhat different sex ratio. Each day twice as many females as males hatched out, so that of the 340 individuals obtained from this pair, 222 were females and 118 were males, making a sex ratio of 2 : 1.

The fifth pair proved to be even more unusual than the fourth in its excess of females for each day two, three and even four times as many females as males would hatch out. The total

number of the offspring of this pair was 439, of which 308 were females and 131 males, making a sex ratio of 2.3 : 1.

When this unusual ratio was first noticed, at the suggestion of Professor Morgan, some of the flies that hatched out were mated, the sisters with their brothers, in order to see whether the peculiar ratio of the parents would be transmitted to any of the children. Sixty-three pairs were made up and kept in good condition, each female being allowed to lay all of her eggs, and the offspring of each carefully separated according to sex and counted. Out of the 63 pairs, 26 showed various high and in some cases unexpected sex ratios, while 37 gave a normal ratio. The results are shown in the following table:

TABLE II.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	312	3	104 : 1
No. 2	169	5	34 : 1
No. 3	276	26	10 : 1
No. 4	291	54	5 : 1
No. 5	147	32	4 : 1
No. 6	87	27	3 : 1
No. 7	86	30	3 : 1
No. 8	225	128	2 : 1
No. 9	135	61	2 : 1
No. 10	163	62	2 : 1
No. 11	289	148	2 : 1
No. 12	121	65	2 : 1
No. 13	367	166	2 : 1
No. 14	236	108	2 : 1
No. 15	151	71	2 : 1
No. 16	254	120	2 : 1
No. 17	174	85	2 : 1
No. 18	195	88	2 : 1
No. 19	100	51	2 : 1
No. 20	121	61	2 : 1
No. 21	119	54	2 : 1
No. 22	169	65	2 : 1
No. 23	195	84	2 : 1
No. 24	176	79	2 : 1
No. 25	285	135	2 : 1
No. 26	236	108	2 : 1

One of the first questions that arose was, whether the male or the female was causing the peculiar ratio and to examine this question the following experiments were carried out. The male parent of the best line showing a ratio of 104 : 1 died before I had a chance to breed him with other females, so the father of the

line showing the next best ratio 34 : 1 was used instead. He was mated with five wild females, great care being taken to use only virgin females, with the following results:

TABLE III.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	129	85	1 : 1
No. 2	165	173	1 : 1
No. 3	111	103	1 : 1
No. 4	120	110	1 : 1
No. 5	90	91	1 : 1

The father of another good line with a sex ratio of 10 : 1 was also crossed with two virgin wild females and the following results obtained:

TABLE IV.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	89	103	1 : 1
No. 2	47	50	1 : 1

Eight males were taken promiscuously from the four lines showing the four best sex ratios and crossed with virgin wild females, with results as follows:

TABLE VI.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	220	192	1 : 1
No. 2	147	127	1 : 1
No. 3	167	171	1 : 1
No. 4	103	80	1 : 1
No. 5	134	147	1 : 1
No. 6	178	148	1 : 1
No. 7	112	105	1 : 1
No. 8	115	119	1 : 1

All of these results seem to show conclusively that the abnormal sex ratio is not caused or influenced by the male and it seems probable that it is to the female we must look for a further explanation. This conclusion, therefore, eliminates one of the possible explanations for an excess of females, namely, that the male-producing sperm are non-functional.

Other experiments were carried on at the same time with the female children of the four best lines, to see if the female was causing the unusual ratios. Twelve of the virgin females were crossed with wild males, with these results:

TABLE VIII.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	112	43	2+ : 1
No. 2	201	129	1.6 : 1
No. 3	110	108	1 : 1
No. 4	84	38	2 : 1
No. 5	211	103	2 : 1
No. 6	248	121	2 : 1
No. 7	189	191	1 : 1
No. 8	211	138	1.5 : 1
No. 9	57	24	2 : 1
No. 10	152	110	1 : 1
No. 11	147	61	2 : 1
No. 12	156	86	2 : 1

Thus it is shown that it is the female that produces the abnormal sex ratios, just how, is not apparent at present.

One hundred and six of the females from the best line with the 104 : 1 ratio were crossed with wild males in order to see whether any of these children had inherited an abnormal sex ratio from their mother and whether the ratio in any case would be as high as that of the mother. The results are as follows in Table IX.

Thus, although none of the children[1] inherited as high a ratio as their mother showed, at the same time almost all of them inherited a ratio above normal, the majority showing that of their grandmother.

Another generation was obtained by mating 119 of the virgin females taken from the lines showing a 3 : 1 ratio to wild males, in order to see whether the abnormal ratio still persisted and if the 104 : 1 ratio could be recovered again. The general results are as follows:

11 females showed a sex ratio of 3 : 1
48 females showed a sex ratio of 2 : 1
60 females showed a sex ratio of 1 : 1

The exact figures are given in Table X.

[1] Eight females died.

TABLE IX.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	144	56	(−3) : 1
No. 2	282	99	3 : 1
No. 3	323	112	3 : 1
No. 4	107	35	3 : 1
No. 5	201	67	3 : 1
No. 6	150	49	3 : 1
No. 7	219	75	3 : 1
No. 8	220	76	3 : 1
No. 9	106	39	3 : 1
No. 10	289	102	(−3) : 1
No. 11	277	139	2 : 1
No. 12	177	96	(−2) : 1
No. 13	316	160	2 : 1
No. 14	248	102	2 : 1
No. 15	250	101	2 : 1
No. 16	207	113	2 : 1
No. 17	173	84	2 : 1
No. 18	276	133	2 : 1
No. 19	113	64	(−2) : 1
No. 20	167	79	2 : 1
No. 21	173	79	2 : 1
No. 22	213	111	2 : 1
No. 23	120	69	2 : 1
No. 24	193	93	2 : 1
No. 25	164	72	2 : 1
No. 26	195	96	2 : 1
No. 27	204	102	2 : 1
No. 28	251	98	2 : 1
No. 29	189	72	2 : 1
No. 30	129	67	2 : 1
No. 31	209	85	2 : 1
No. 32	144	59	2 : 1
No. 33	139	57	2 : 1
No. 34	201	76	2 : 1
No. 35	109	44	2 : 1
No. 36	191	78	2 : 1
No. 37	239	101	2 : 1
No. 38	148	65	2 : 1
No. 39	129	73	(−2) : 1
No. 40	228	93	2 : 1
No. 41	317	148	2 : 1
No. 42	235	115	2 : 1
No. 43	252	121	2 : 1
No. 44	138	56	2 : 1
No. 45	154	66	2 : 1
No. 46	87	43	2 : 1
No. 47	118	50	2 : 1
No. 48	214	83	2 : 1
No. 49	181	98	2 : 1
No. 50	205	120	(−2) : 1
No. 51	254	104	2 : 1
No. 52	156	85	(−2) : 1
No. 53	211	85	2 : 1
No. 54	175	89	2 : 1
No. 55	178	70	2 : 1
No. 56	145	72	2 : 1
No. 57	123	48	2 : 1
No. 58	191	94	2 : 1
No. 59	187	66	2 : 1

TABLE IX—*Continued.*

Pair.	No. of Females.	No. of Males.	Ratio.
No. 60	167	64	2 : 1
No. 61	117	57	2 : 1
No. 62	130	63	2 : 1
No. 63	322	140	2 : 1
No. 64	129	62	2 : 1
No. 65	229	111	2 : 1
No. 66	300	123	2 : 1
No. 67	269	143	(−2) : 1
No. 68	286	133	2 : 1
No. 69	284	126	2 : 1
No. 70	240	124	2 : 1
No. 71	92	40	2 : 1
No. 72	273	118	2 : 1
No. 73	197	98	2 : 1
No. 74	70	35	2 : 1
No. 75	243	103	2 : 1
No. 76	168	63	2 : 1
No. 77	116	56	2 : 1
No. 78	210	102	2 : 1
No. 79	243	110	2 : 1
No. 80	237	108	2 : 1
No. 81	149	65	2 : 1
No. 82	124	56	2 : 1
No. 83	82	41	2 : 1
No. 84	64	28	2 : 1
No. 85	133	64	2 : 1
No. 86	122	57	2 : 1
No. 87	96	45	2 : 1
No. 88	97	54	2 : 1
No. 89	56	25	2 : 1
No. 90	35	17	2 : 1
No. 91	49	26	2 : 1
No. 92	165	104	(1+) : 1
No. 93	193	118	(1+) : 1
No. 94	210	216	1 : 1
No. 95	133	85	(1+) : 1
No. 96	150	140	1 : 1
No. 97	178	107	(1+) : 1
No. 98	71	44	1 : 1

TABLE X.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	143	49	3 : 1
No. 2	164	59	3 : 1
No. 3	124	43	3 : 1
No. 4	149	50	3 : 1
No. 5	118	39	3 : 1
No. 6	139	49	3 : 1
No. 7	143	45	3 : 1
No. 8	144	52	3 : 1
No. 9	111	39	3 : 1
No. 10	87	30	3 : 1
No. 11	91	33	3 : 1
No. 12	140	63	2 : 1
No. 13	36	17	2 : 1
No. 14	140	75	2 : 1
No. 15	141	52	2(+) : 1
No. 16	83	40	2 : 1
No. 17	66	36	2 : 1
No. 18	35	19	2 : 1
No. 19	150	68	2 : 1
No. 20	95	50	2 : 1
No. 21	176	70	2 : 1
No. 22	182	90	2 : 1
No. 23	50	23	2 : 1
No. 24	34	16	2 : 1
No. 25	83	44	2 : 1
No. 26	119	51	2 : 1
No. 27	110	40	2(+) : 1
No. 28	96	50	2 : 1
No. 29	133	74	2 : 1
No. 30	146	76	2 : 1
No. 31	51	23	2 : 1
No. 32	138	53	2 : 1
No. 33	147	71	2 : 1
No. 34	126	62	2 : 1
No. 35	153	79	2 : 1
No. 36	104	46	2 : 1
No. 37	103	44	2 : 1
No. 38	79	42	2 : 1
No. 39	147	62	2 : 1
No. 40	79	40	2 : 1
No. 41	162	77	2 : 1
No. 42	92	45	2 : 1
No. 43	98	51	2 : 1
No. 44	122	60	2 : 1
No. 45	96	52	2 : 1
No. 46	118	46	2 : 1
No. 47	133	55	2 : 1
No. 48	68	33	2 : 1
No. 49	72	37	2 : 1
No. 50	76	34	2 : 1
No. 51	105	55	2 : 1
No. 52	173	89	2 : 1
No. 53	78	40	2 : 1
No. 54	124	46	2(+) : 1
No. 55	106	50	2 : 1
No. 56	89	39	2 : 1
No. 57	139	61	2 : 1
No. 58	102	50	2 : 1
No. 59	90	50	2 : 1
No. 60	117	104	1: 1

TABLE X—*Continued.*

Pair.	No. of Females.	No. of Males.	Ratio.
No. 61	75	55	1 : 1
No. 62	46	56	1 : 1
No. 63	25	25	1 : 1
No. 64	53	55	1 : 1
No. 65	27	36	1 : 1
No. 66	91	78	1 : 1
No. 67	60	59	1 : 1
No. 68	118	86	1 : 1
No. 69	110	66	1(−) : 1
No. 70	61	51	1 : 1
No. 71	44	53	1 : 1
No. 72	114	100	1 : 1
No. 73	103	64	1(−) : 1
No. 74	122	85	1 : 1
No. 75	82	57	1 : 1
No. 76	122	122	1 : 1
No. 77	109	88	1 : 1
No. 78	142	131	1 : 1
No. 79	128	132	1 : 1
No. 80	133	129	1 : 1
No. 81	97	99	1 : 1
No. 82	114	96	1 : 1
No. 83	97	77	1 : 1
No. 84	111	98	1 : 1
No. 85	102	107	1 : 1
No. 86	55	46	1 : 1
No. 87	93	87	1 : 1
No. 88	92	63	1 : 1
No. 89	98	90	1 : 1
No. 90	118	98	1 : 1
No. 91	136	141	1 : 1
No. 92	109	96	1 : 1
No. 93	118	109	1 : 1
No. 94	105	85	1 : 1
No. 95	136	104	1 : 1
No. 96	121	101	1 : 1
No. 97	133	159	1 : 1
No. 98	164	94	1 : 1
No. 99	72	77	1 : 1
No. 100	84	81	1 : 1
No. 101	98	60	1(−) : 1
No. 102	93	55	1(−) : 1
No. 103	106	106	1 : 1
No. 104	94	73	1 : 1
No. 105	80	68	1 : 1
No. 106	66	54	1 : 1
No. 107	81	85	1 : 1
No. 108	71	40	1(−) : 1
No. 109	82	80	1 : 1
No. 110	108	89	1 : 1
No. 111	91	49	1(−) : 1
No. 112	102	100	1 : 1
No. 113	93	106	1 : 1
No. 114	45	67	1 : 1
No. 115	101	79	1 : 1
No. 116	93	92	1 : 1
No. 117	88	104	1 : 1
No. 118	84	85	1 : 1
No. 119	67	70	1 : 1

Two other generations have been carried through with these results:

From 49 females mated

1 showed a ratio of 4 : 1 — 3 showed a ratio of 3 : 1
12 showed a ratio of 2 : 1 — 33 showed a ratio of 1 : 1

The exact figures are:

TABLE XI.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	92	22	4 : 1
No. 2	97	33	3 : 1
No. 3	62	16	3 : 1
No. 4	139	50	3 : 1
No. 5	105	48	2 : 1
No. 6	95	41	2 : 1
No. 7	147	66	2+ : 1
No. 8	148	59	2+ : 1
No. 9	66	33	2 : 1
No. 10	46	21	2 : 1
No. 11	174	65	2+ : 1
No. 12	76	38	2 : 1
No. 13	172	79	2+ : 1
No. 14	74	39	2 : 1
No. 15	110	54	2 : 1
No. 16	79	41	2 : 1
No. 17	117	78	1+ : 1
No. 18	138	130	1 : 1
No. 19	96	91	1 : 1
No. 20	110	98	1 : 1
No. 21	64	60	1 : 1
No. 22	152	87	1+ : 1
No. 23	48	35	1 : 1
No. 24	162	165	1 : 1
No. 25	180	138	1+ : 1
No. 26	73	79	1 : 1
No. 27	91	89	1 : 1
No. 28	67	68	1 : 1
No. 29	117	78	1+ : 1
No. 30	148	152	1 : 1
No. 31	155	110	1+ : 1
No. 32	52	39	1 : 1
No. 33	62	46	1+ : 1
No. 34	69	44	1+ : 1
No. 35	135	114	1 : 1
No. 36	126	95	1 : 1
No. 37	78	45	1+ : 1
No. 38	100	71	1 : 1
No. 39	64	70	1 : 1
No. 40	70	64	1 : 1
No. 41	46	59	1 : 1
No. 42	136	95	1+ : 1
No. 43	63	40	1+ : 1
No. 44	61	67	1 : 1
No. 45	74	73	1 : 1
No. 46	107	76	1 : 1
No. 47	37	38	1 : 1
No. 48	96	77	1 : 1
No. 49	91	107	1 : 1

From 25 females mated with wild males

1 showed a ratio of 3 : 1
10 showed a ratio of 2 : 1
14 showed a ratio of 1 : 1

The exact figures are:

TABLE XII.

Pair.	No. of Females.	No. of Males.	Ratio.
No. 1	91	31	3 : 1
No. 2	69	33	2 : 1
No. 3	123	51	2 : 1
No. 4	90	47	2 : 1
No. 5	116	59	2 : 1
No. 6	104	41	2+ : 1
No. 7	90	45	2 : 1
No. 8	59	24	2 : 1
No. 9	58	26	2 : 1
No. 10	60	25	2 : 1
No. 11	53	27	2 : 1
No. 12	40	46	1 : 1
No. 13	39	57	1 : 1
No. 14	86	57	1 : 1
No. 15	96	87	1 : 1
No. 16	70	61	1 : 1
No. 17	51	48	1 : 1
No. 18	69	58	1 : 1
No. 19	59	71	1 : 1
No. 20	121	78	1+ : 1
No. 21	59	69	1 : 1
No. 22	68	57	1 : 1
No. 23	52	64	1 : 1
No. 24	94	83	1 : 1
No. 25	56	43	1 : 1

The results of these last generations seem to indicate that the unusual sex ratio is gradually disappearing, to judge from the number of individuals that inherit it, but whether it can be maintained by breeding from certain strains remains to be determined.